Physical Equilibria and all that

Exam Results

Binding Equilibria
Equilibrium Dialysis
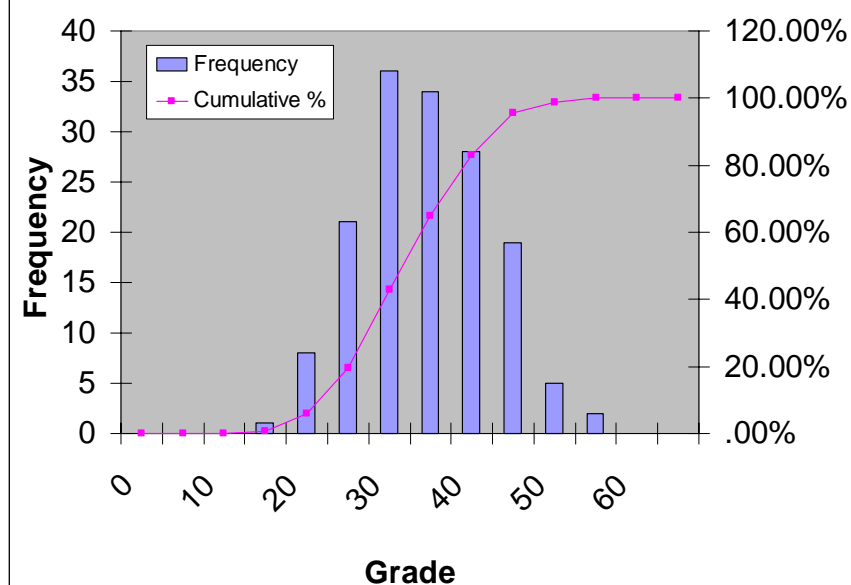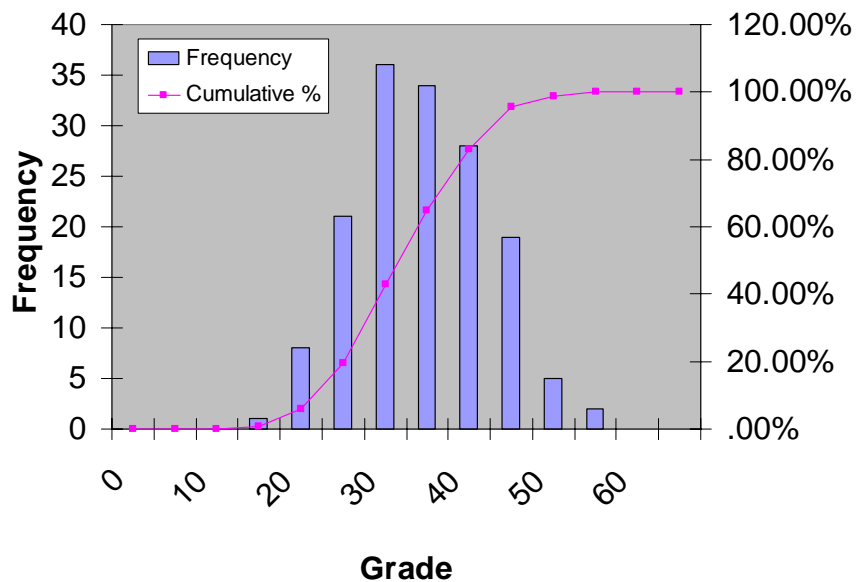Scatchard Plots
Cooperativity
Donnan Effect
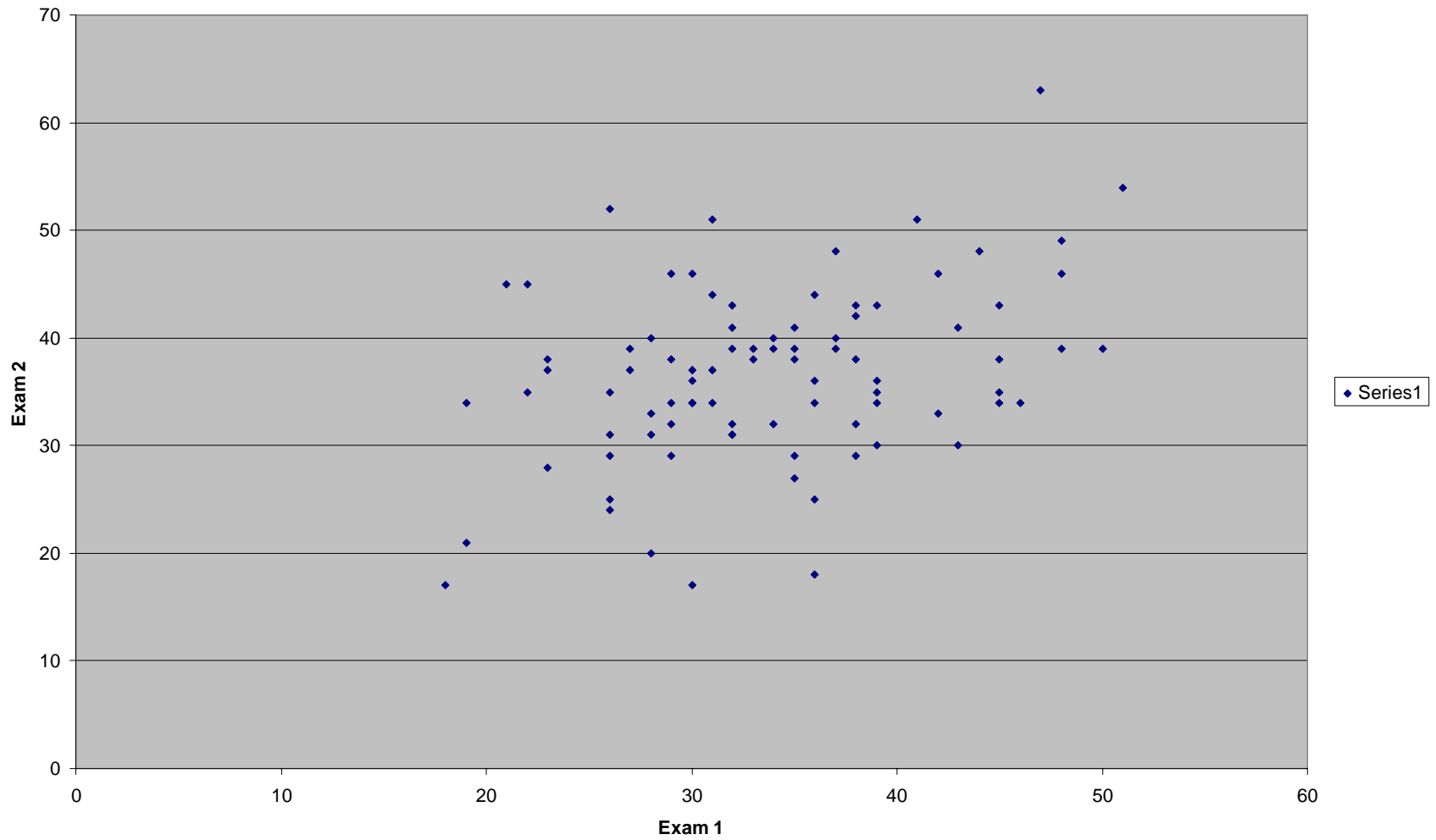
Preliminary Results

Average	32.57792	36.38636
SD	8.021036	8.726274
MAX	52	63
MIN	15	9

Exam1 vs Exam2

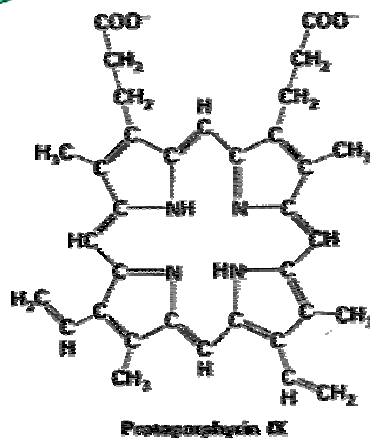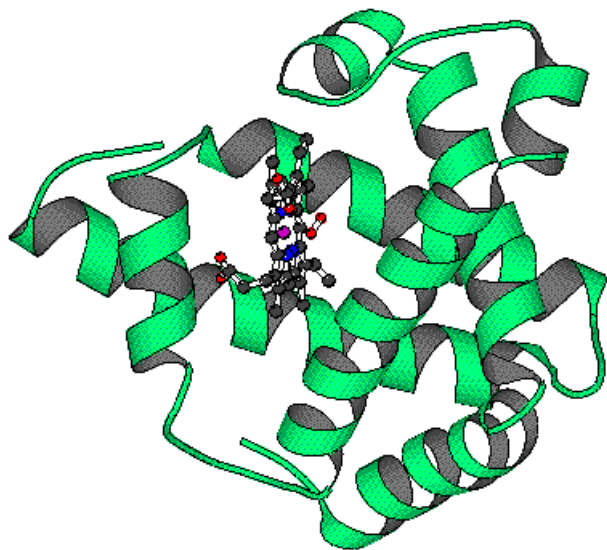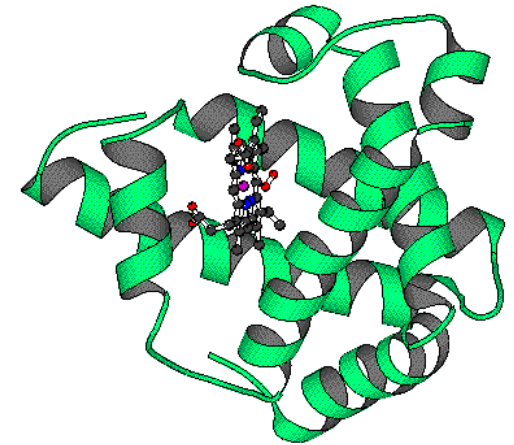We were talking about binding equilibria and their effect on partitionings.

Myoglobin and Hemoglobin help to partition oxygen into the blood stream. However, the binding of oxygen to these molecules is very different.

Protoporphyrin IX

Rossi, Fanelli, Antonini (1958(
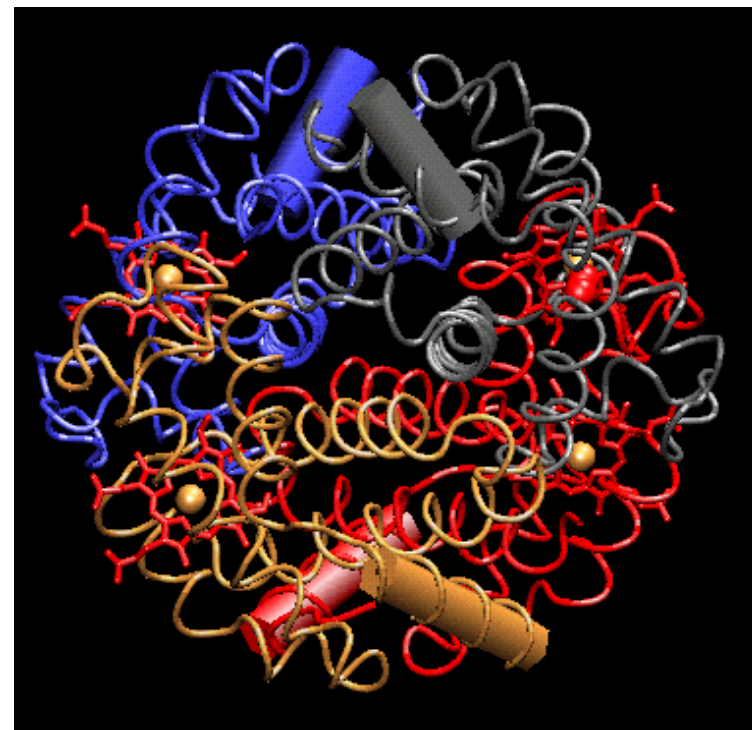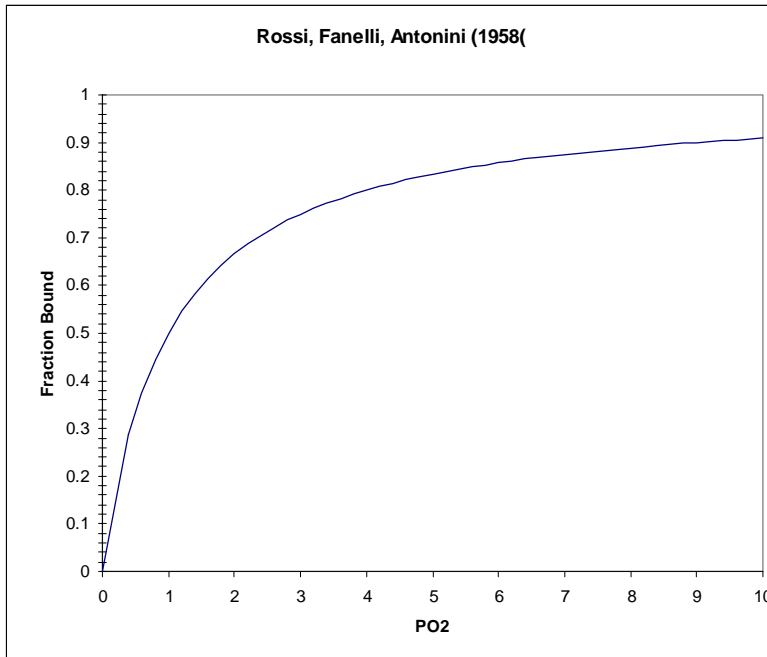
Myoglobin + O_2 ⟷ Myo-O_2

Total Myoglobin = Myo_o = Myoglobin + Myo-O_2

$K = [O_2][Myoglobin]/[Myo-O_2]$

$Myo_o = K[Myo-O_2]/[O_2] + [Myo-O_2]$ $[Myo-O2]/Myo_o = [O_2]/(K + [O_2])$

This is for binding of one molecule of O_2 to one molecule of Myoglobin.

The binding constant for O_2 to myoglobin, or any ligand to a protein can be measured via equilibrium dialysis.

For the myoglobin experiment the apparatus might look like:

O$_2$

P$_{O2}$

O$_2$

Mb O$_2$

Dialysis Membrane
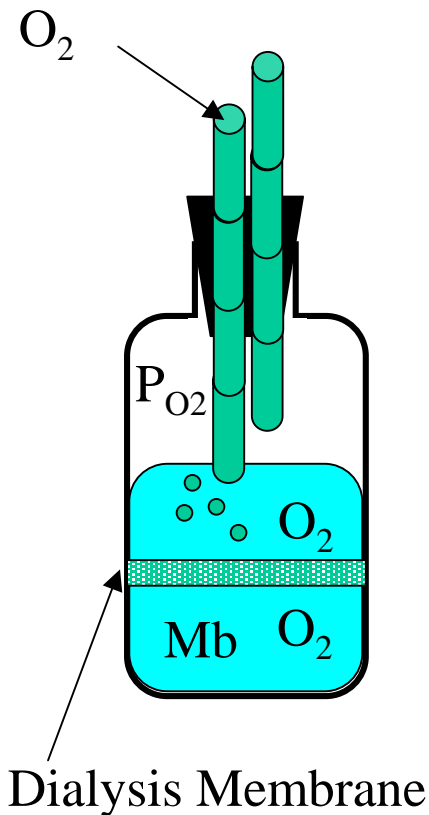
Here we bubble in O_2 to maintain a particular partial pressure above the solution.

Henry's Law gives the concentration of O_2 in the aqueous buffer solution.

$$c_{O2}(out)=[O_2]$$

We also know

$$c_M = [Mb] + [Mb\ O_2]$$
$$c_{O2}(in) = [O_2] + [Mb\ O_2]$$
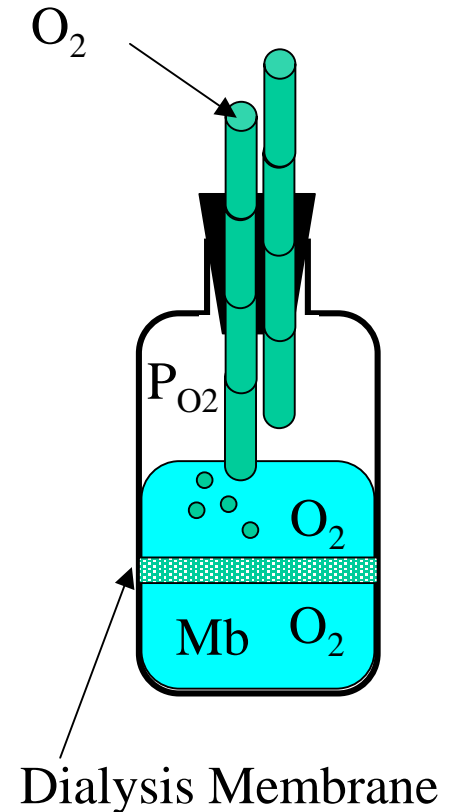$$c_{O2}(bound)= c_{O2}(in)-c_{O2}(out) = [Mb\ O_2]$$

$$c_M \quad = \quad [Mb] + [Mb\ O_2]$$
$$c_{O2}(in) \quad = \quad [O_2] \ + [Mb\ O_2]$$
$$c_{O2}(bound) = \quad c_{O2}(in) - c_{O2}(out) = [Mb\ O_2]$$

O_2

So now we have the concentration of bound oxygen as a function of two measurables!

The equilibrium constant is then easily calculated:

P_{O2}

O_2

Mb O_2

Dialysis Membrane

$$K \quad = \quad [Mb\ O_2]/[Mb][O_2]$$

$$= \quad c_{O2}(bound)/(c_M - c_{O2}(bound))* \ c_{O2}(out)$$

$$K = [\text{Mb } O_2]/[\text{Mb}][O_2]$$

$$= c_{O2}(\text{bound})/(c_M - c_{O2}(\text{bound}))* c_{O2}(\text{out})$$

This form for the equilibrium constant is a little confusing but can be rewritten.

We introduce

ν = Number of ligands bound per macromolecule
$= c_{ligand}(\text{bound})/c_M$

Using this notation

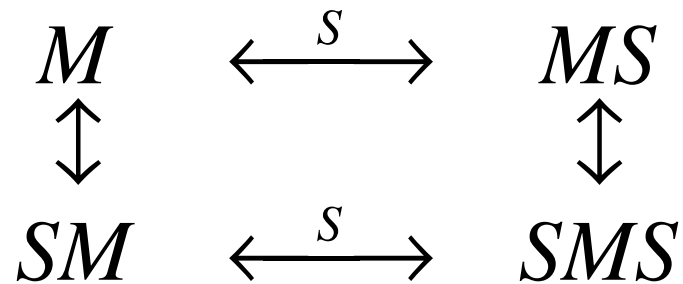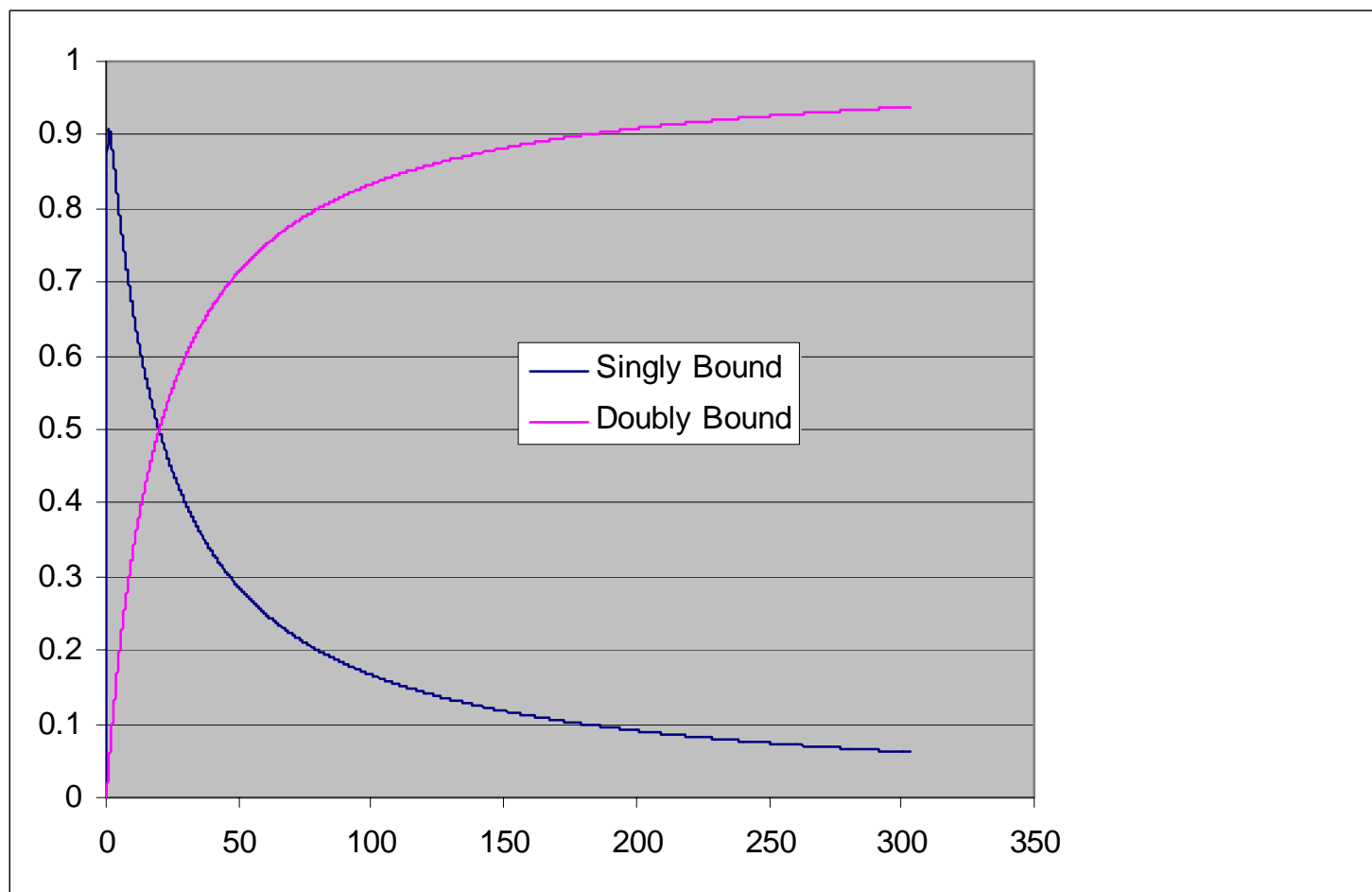$$K = \nu/((1-\nu)* c_L(\text{out})) = \nu/((1-\nu)* [L])$$

Rearranging:

$$\boxed{\nu/[L] = K* (1-\nu)}$$

But we have been assuming that only one molecule of ligand binds to the macromolecule!

What if two could bind?

$$M \quad \xrightarrow{\quad S \quad} \quad MS$$
$$\updownarrow \qquad\qquad\qquad \updownarrow$$
$$SM \quad \xrightarrow{\quad S \quad} \quad SMS$$

Here there are formally four equilibrium constants but lets assume they are all equal.

Can we do that?
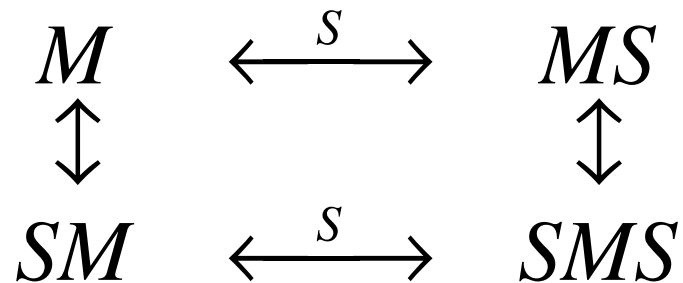
$$M \xleftrightarrow{\quad S \quad} MS$$
$$\updownarrow \qquad\qquad \updownarrow$$
$$SM \xleftrightarrow{\quad S \quad} SMS$$

Here the fraction bound is

(given K= [MS]/[M][S] = [SMS]/[MS][S]=[SMS]/[SM][S]

f   =   ([MS]+[SM]+[SMS])/c_m
    =   (c_m-[M])/ c_m = (1- [M]/cm)

c_m= [M]+[MS]+[SM]+[SMS]= [M]+K[M][S]+K[M][S]+K[MS][S]
    = [M] (1 + 2*K[S] + K^2[S]^2)

so

f= 1- 1/(1 + 2*K[S] + K^2[S]^2)

$$M \quad \xleftrightarrow{\quad S \quad} \quad MS$$

$$\updownarrow \qquad\qquad\qquad \updownarrow$$
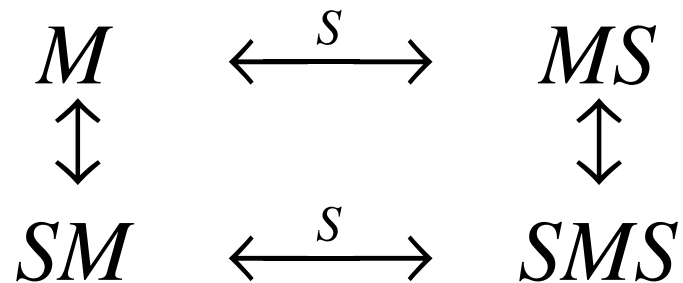
$$SM \quad \xleftrightarrow{\quad S \quad} \quad SMS$$

The NUMBER of ligands bound per macromolecule is

$\quad\quad$ ν $\quad\quad$ = $\quad\quad$ 1*fraction in singly bound form + 2* doubly bound

c_m= [M]+[MS]+[SM]+[SMS] = [MS]/([S]*K) +[MS]+[SM]+[SMS]

But we know [SM]=[MS] by symmetry

c_m = (1+1/([S]*K))[MS] +[MS] + K[MS][S]
$\quad$ = ((1+1/([S]*K)) + 1 + K[S]) [MS]
So
[MS]/c_m= 1/(2+K*[S]+1/(K[S]))= K[S]/(2K[S] + K^2[S]^2 + 1)

$$M \quad \xleftrightarrow{\quad S \quad} \quad MS$$

$$\updownarrow \qquad\qquad\qquad \updownarrow$$
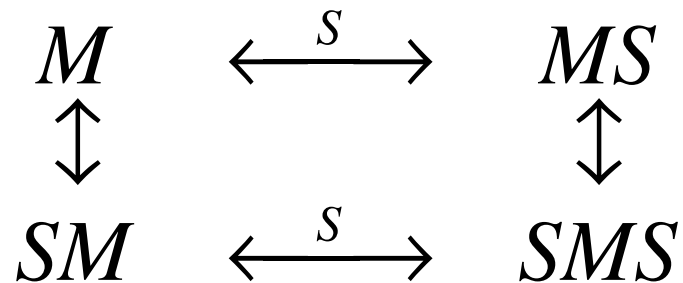
$$SM \quad \xleftrightarrow{\quad S \quad} \quad SMS$$

Similarly

$c_m = [M]+[MS]+[SM]+[SMS] = [MS]/([S]*K) +[MS]+[SM]+[SMS]$

$c_m = (2+1/([S]*K))\,[MS] + [SMS]$

$\quad = (2+1/([S]*K))\,([SMS]/K[S]) + [SMS]$

So

$[SMS]/c_m = K^2[S]^2/(2K^2[S]^2 +1)$

So the concentration of S bound is

$2*[MS]+2*[SMS]= 2*c_m*(K[S]/(2K[S] + K^2[S]^2 + 1) + K^2[S]^2/(2K^2[S]^2 +1))$

What this means is that ν can vary between 0 and 2. Thus, we rewrite our equation
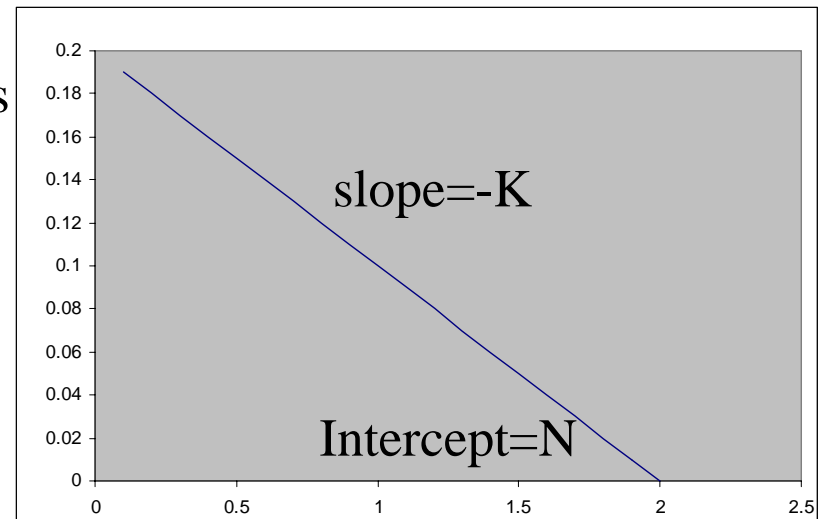
$$(\nu/N)/[L] = K*(1-(\nu/N))$$

where N is the number of equivalent binding sites for ligand.

A simple rearrangement yields:

$$(\nu)/[L] = K*(N-\nu)$$

Scatchard Equation

Plotting (ν)/[L] vs. ν given a plot as follows

slope=-K

Intercept=N

Going back to myoglobin we find

Binding Isotherm

Rossi, Fanelli, Antonini (1958(

Fraction Bound vs PO2

Scatchard Plot

10ºC

30ºC

35ºC
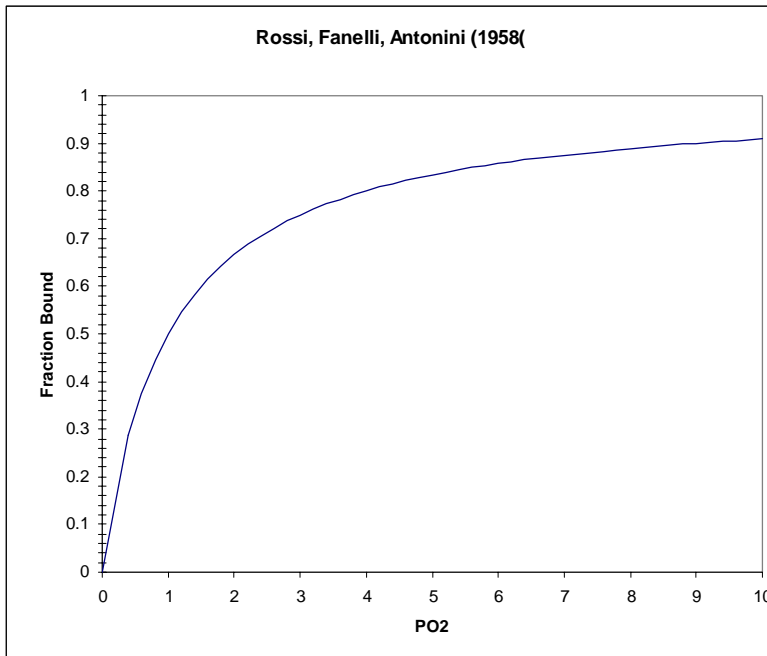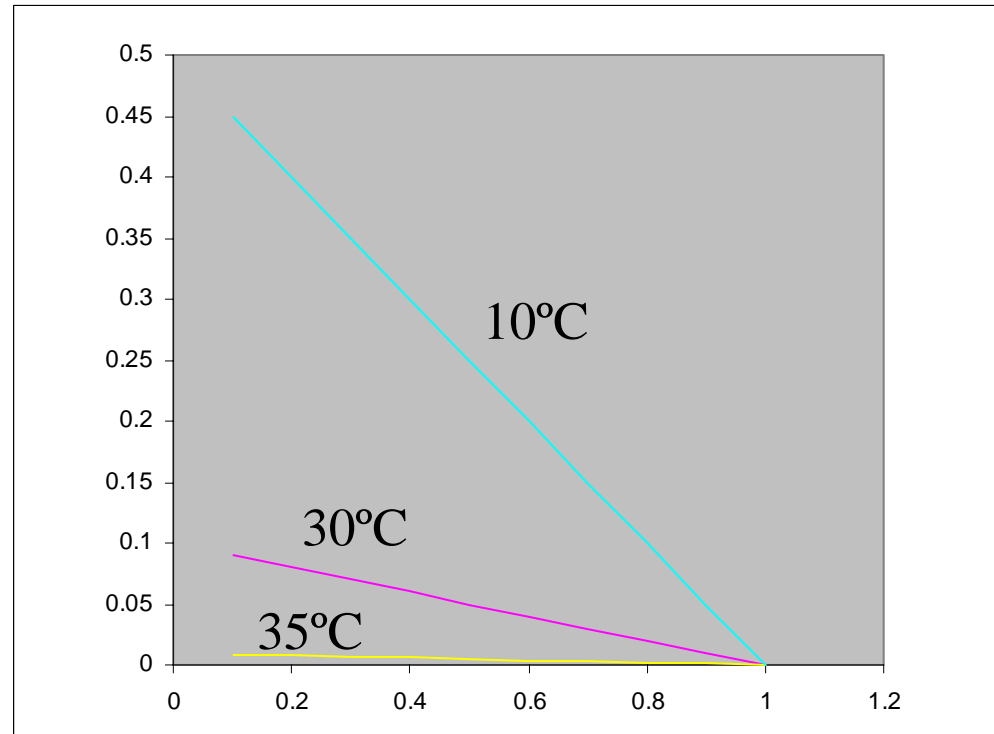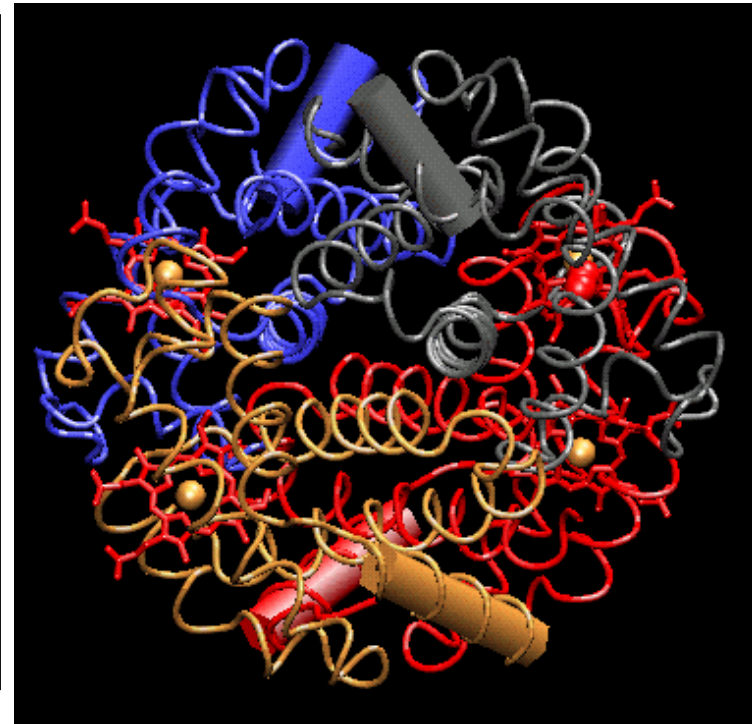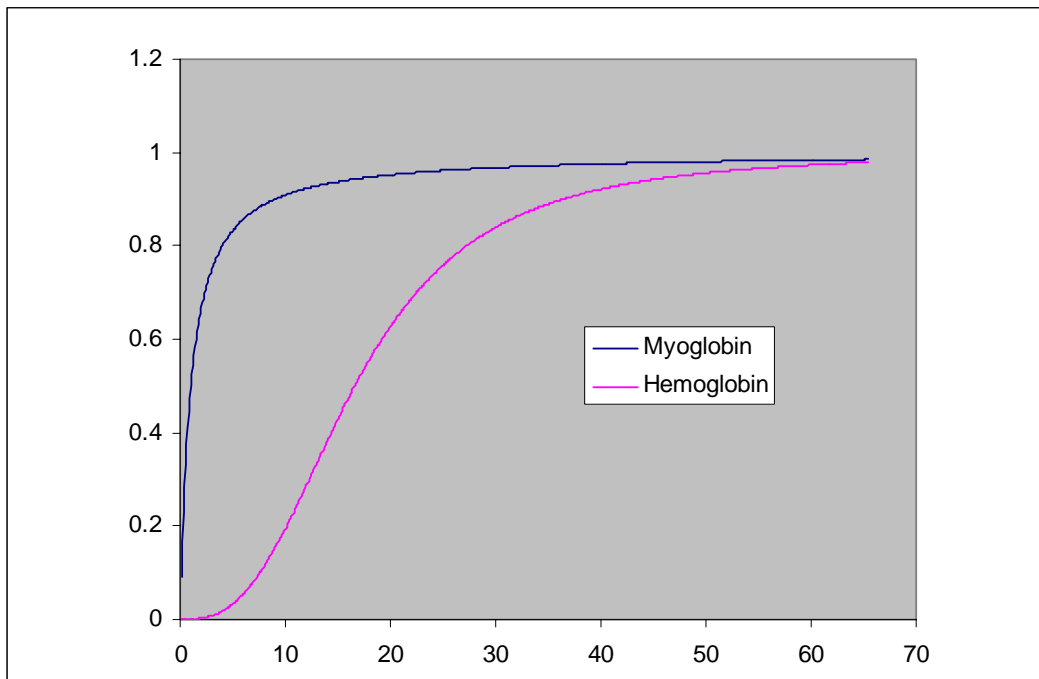
Now for hemoglobin we get a very different curve for the binding isotherm.

It *will not* yield a linear Scatchard plot!

This is *not* due to the fact that hemoglobin has 4 sites for binding!
Rather it is due to interactions among binding sites!

There were a number of attempt to explain this phenomenon. One of the first successful ventures was Hill.

He noted that in terms of the fraction of bound enzyme:

$$(\nu/N)/[L] = K*(1-(\nu/N))$$

$$f/(1-f) = K*[L]$$

For non-interacting sites. Hill proposed the "Concerted Action" model in which n molecules of ligand bind all at once (infinite cooperativity).

From this it is easy to derive that

$$f/(1-f) = K*[L]^N$$

This assumes infinite cooperativity. If the cooperativity is less then

$$f/(1-f) = K*[L]^n \quad \text{where } n<N!$$

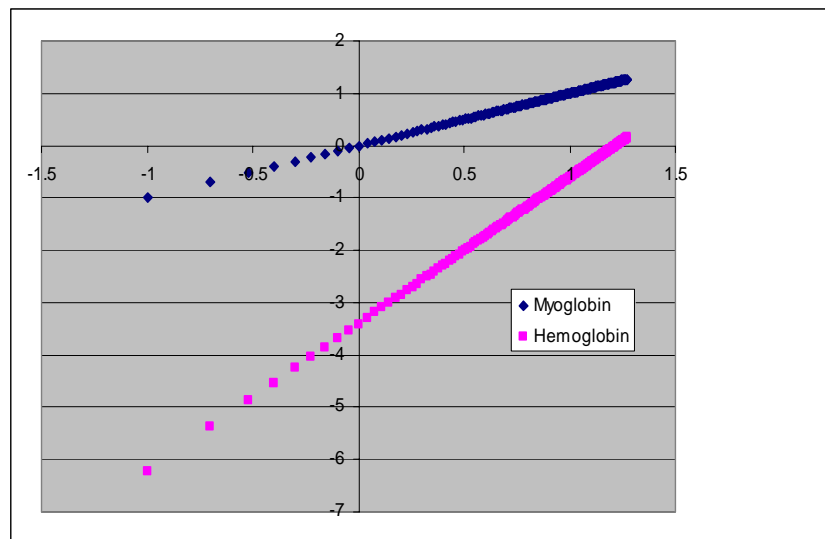This equation, then lends itself to a measure of "cooperativity.

$$f/(1-f) = K*[L]^n \quad \text{where } n<N!$$

If we take logs:

$$\text{Log}(f/1-f) = n\,\text{Log}(L) + \text{Log}(K)$$

So the slope of this curve is a measure of Cooperativity, and the intercept is another measure of K.
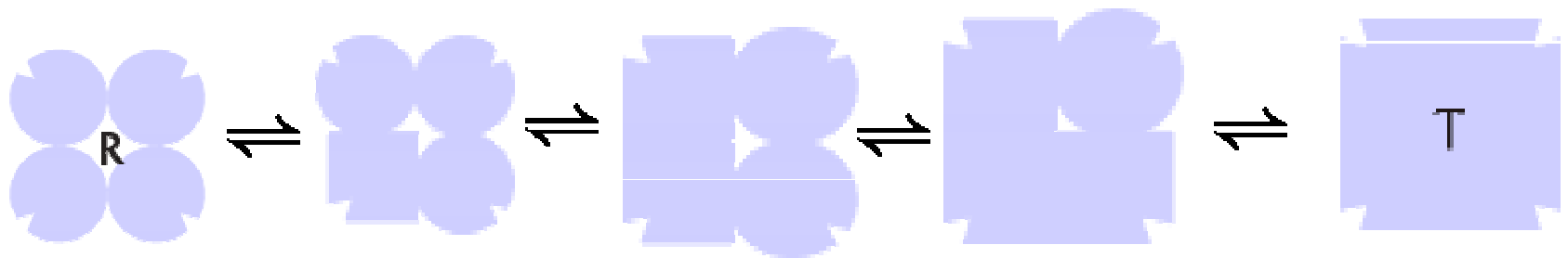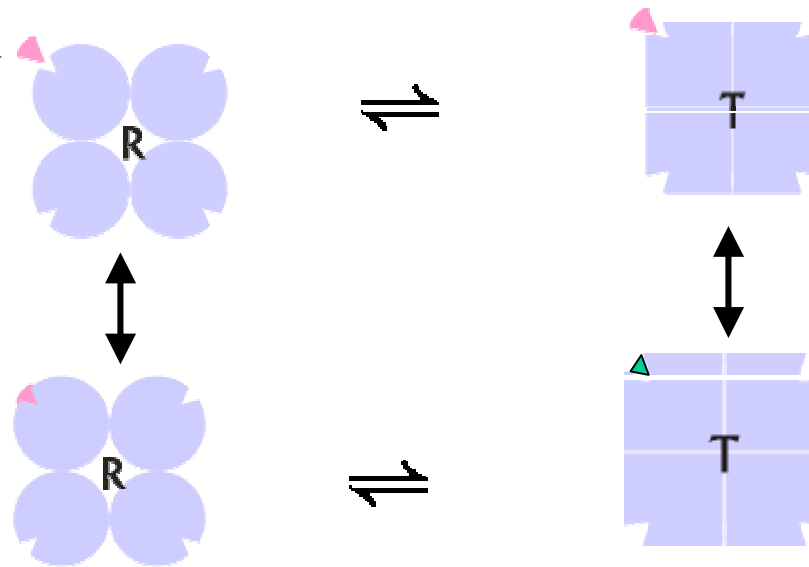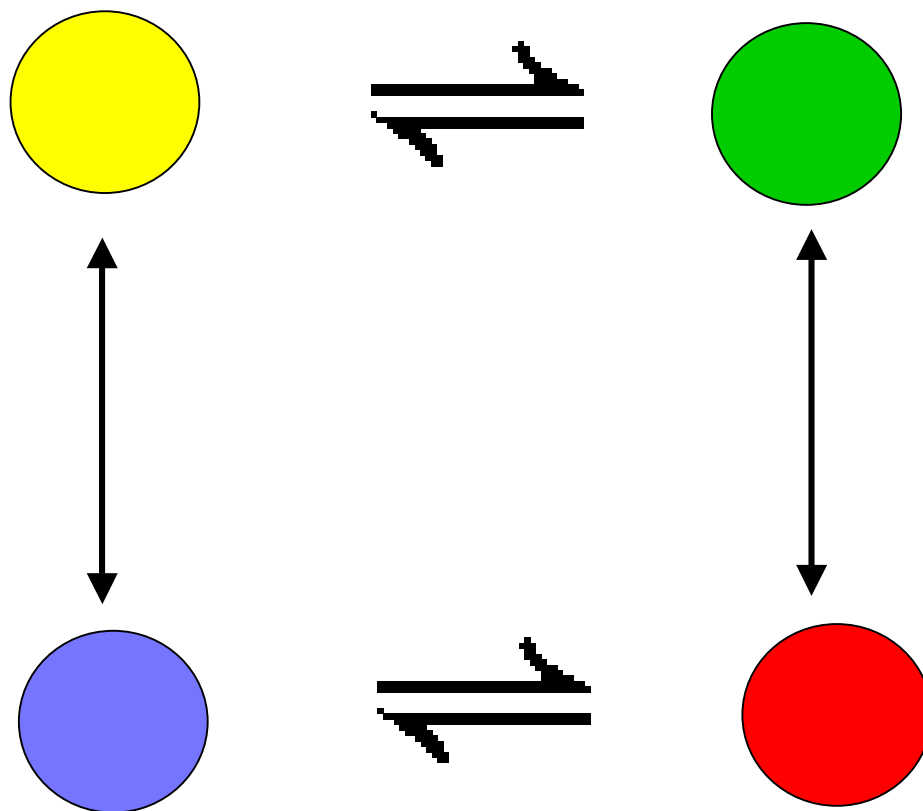
Log(f/(1-f))

Log(P$_{O2}$)
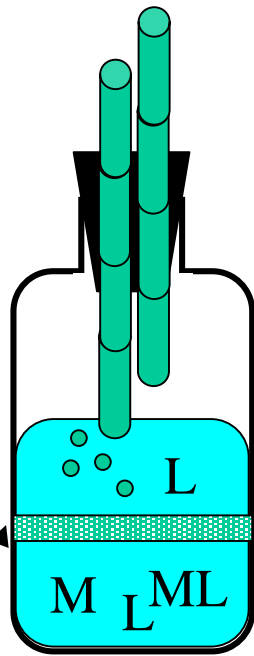
Monod-Changeux-Wyman

Koshland-Nemethy-Filmer

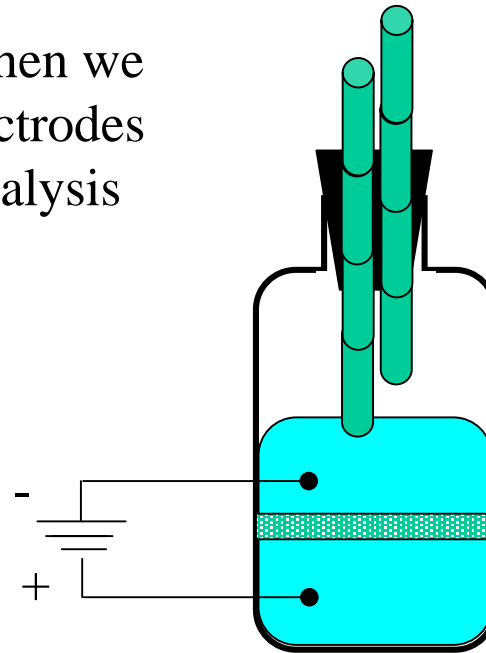Can all the equilibrium constants be independent?

So far we have been considering these molecules as if they are uncharged.

If charges are involved, as we have considered for non-ideal solutions, then strange and wonderful things can happen.

Consider the case when we simply have two electrodes on either side of a dialysis membrane.

L

M $_L$ML

Dialysis Membrane

What will happen to the permeable ions in this solution?

Negative ions will accumulate near the negative electrode and positive ions will accumulate near the positive electrode.

At equilibrium then, there is an unequal concentration of ions on either side of the membrane!

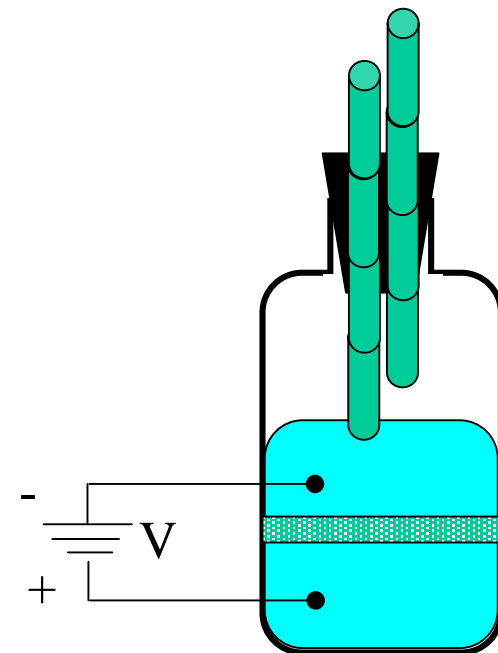The chemical potential must be modified to deal with this effect:

$$\Delta\mu = \Delta\mu° + RT \ln(\mathbf{a}_{ion}(outside)/\mathbf{a}_{ion}(inside))$$

Plus a term indicating the free energy of transfering a charge down a potential gradient:

$$\Delta\mu_{field} = Z F V = Z F (\phi_{in} - \phi_{out})$$

So the total $\Delta\mu$ for the equilibrium described is

$$\Delta\mu = \Delta\mu° + RT \ln(\mathbf{a}_{ion}(outside)/\mathbf{a}_{ion}(inside)) + Z F V$$

0

Now if the macromolecule is charged, now we get a potential due to the fact that the macromolecule is stuck on one side.

We can measure it with the electrodes now.

If we then place ions in solution, negative ions will be attracted to the positive macromolecule leading to an asymmetric distribution of ions at equilibrium!

This occurs even when the interactions are non-specific!

If we can measure the charged ions on either side of the membrane at equilibrium we can calculate the potential different caused by the charged macromolecule

$$0 = RT \ln(\mathbf{a}_{ion}(\text{outside})/\mathbf{a}_{ion}(\text{inside})) + Z F V$$
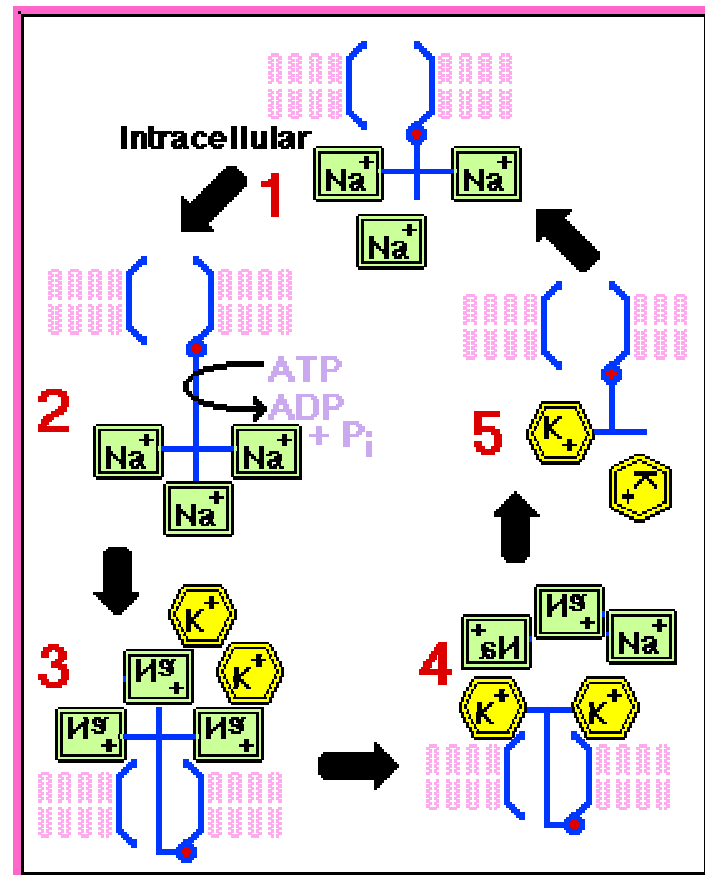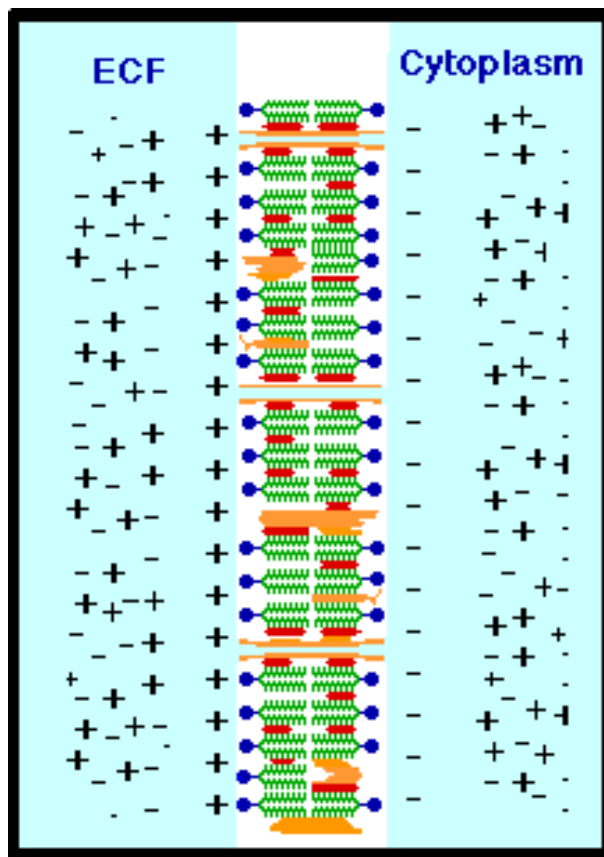
We can solve this for V! This is the ***Donnan potential!***
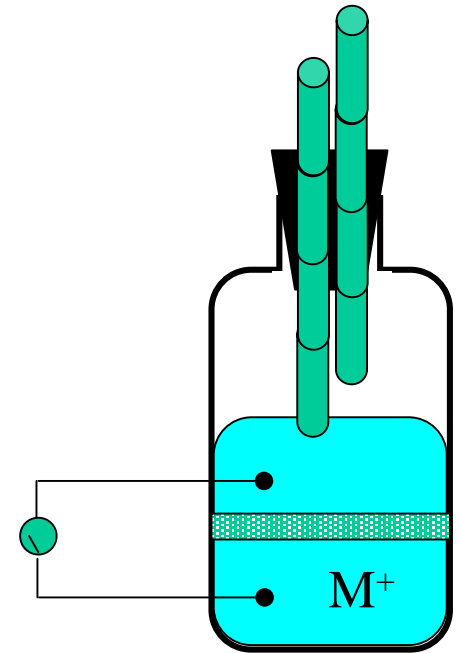
Cells use the Donnan effect to transport molecules across membranes and employ the free energy difference to drive other reactions.

Of course, this is supplemented strongly by active transport.
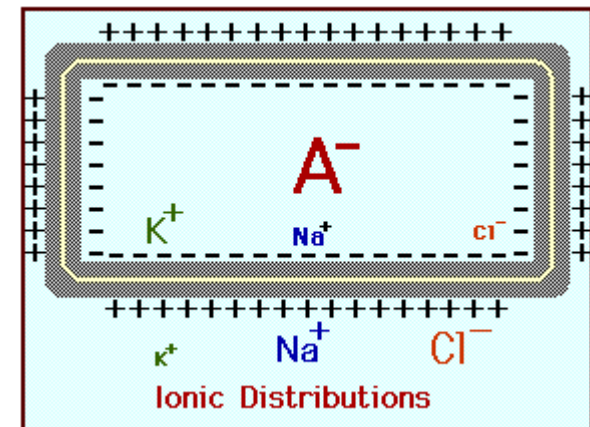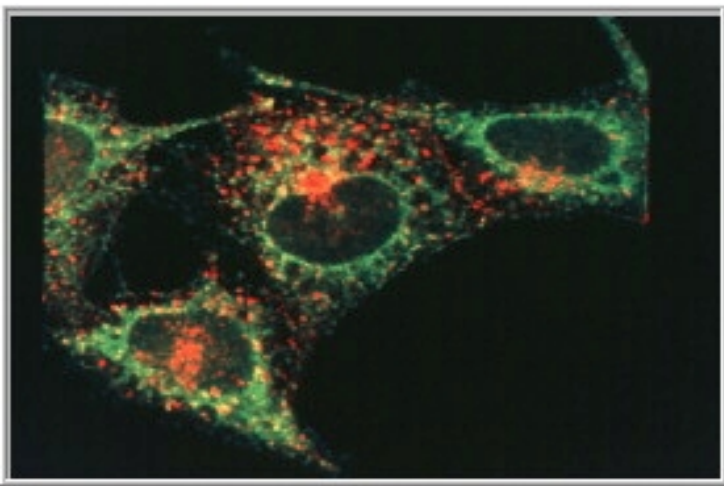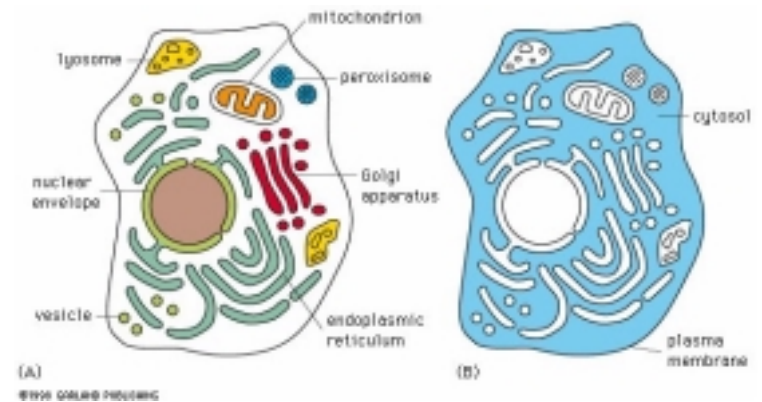
We've mainly been talking about artificial dialysis membranes.

In this case we've been measuring things of biological interest. The same physics is used by the cell to accomplish its own goals.
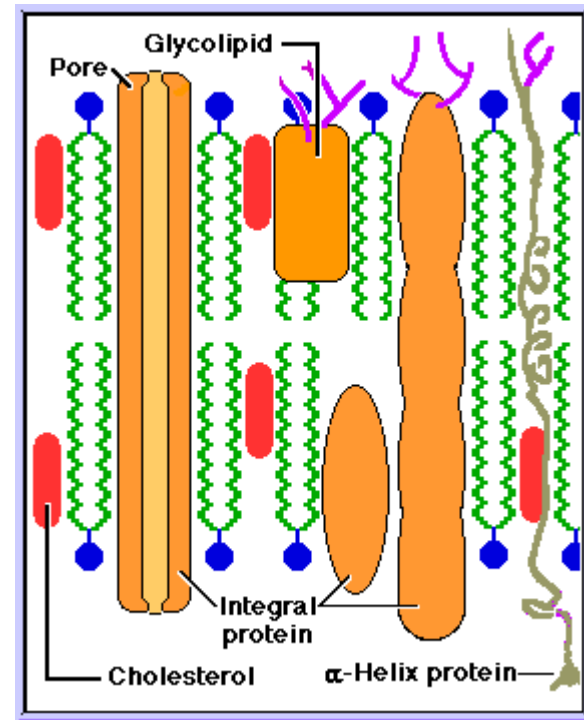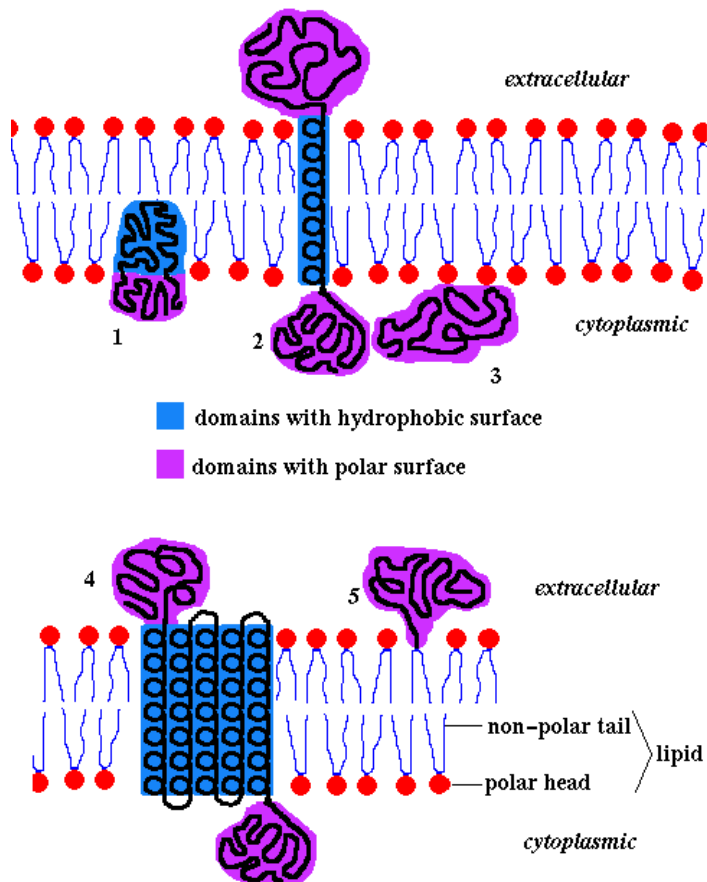
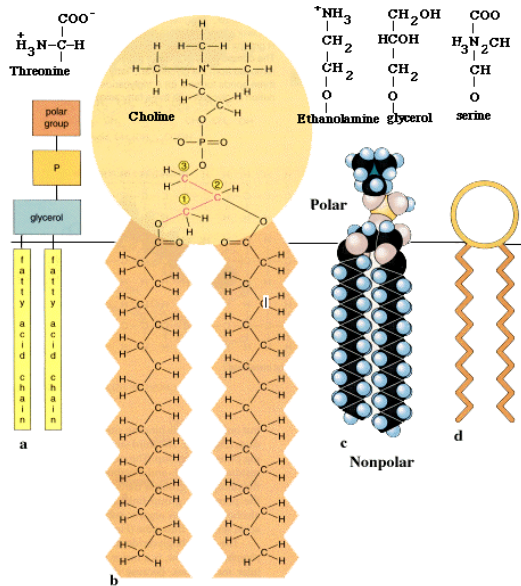Its membranes are mostly lipid though.

Ionic Distributions

These membranes are heterogeneous and impure!

Proteins in the plasma membrane

extracellular

cytoplasmic

1 2 3

■ domains with hydrophobic surface

■ domains with polar surface

4 5

extracellular

non-polar tail
polar head
lipid

cytoplasmic

Glycolipid

Pore

Integral protein
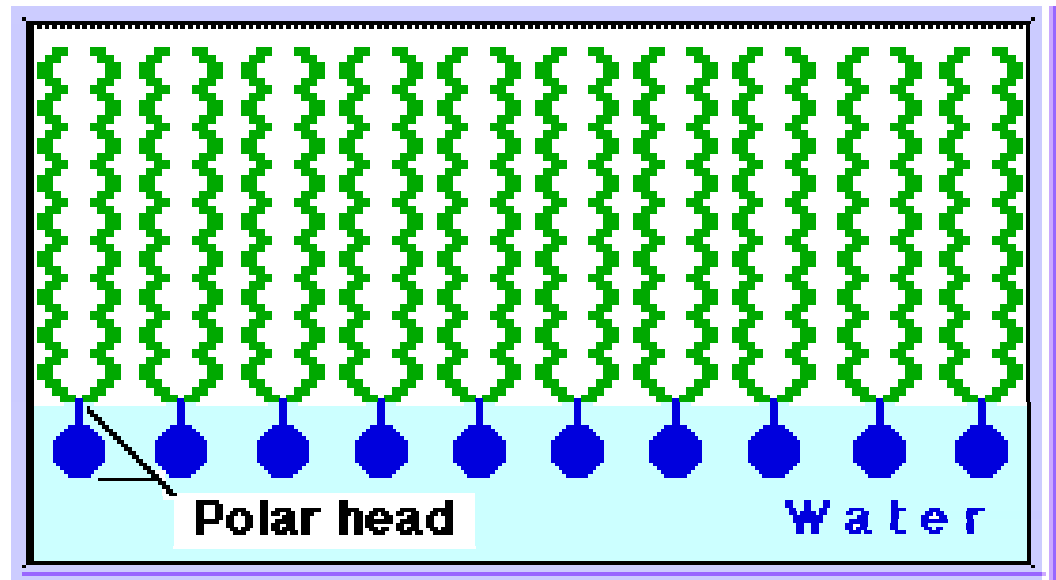
Cholesterol

α-Helix protein

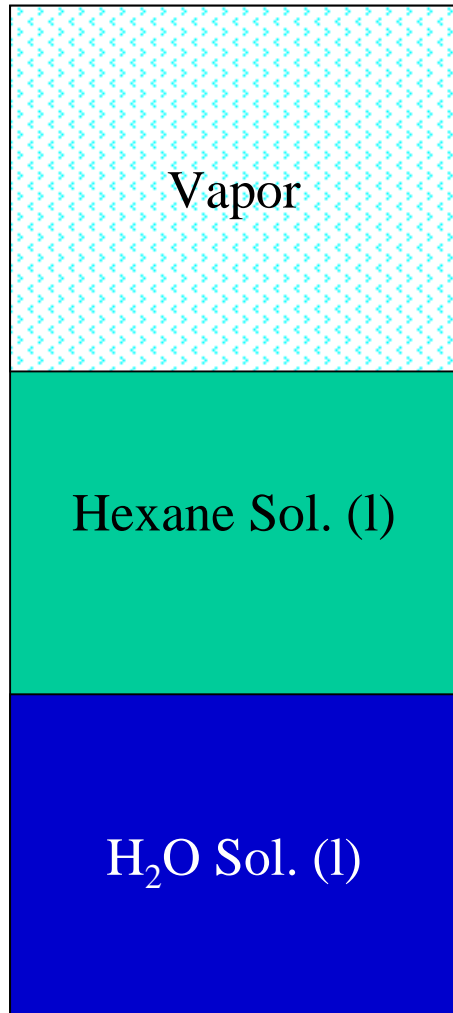Lipids are amphipathic and thus have "water loving" and "water hating" parts.

Thus if you place them in water, they tend to organize at the surface!

There is, of course, a free energy associated with removing them from water. Do you remember the hexane example?

Phase Equilibria

Vapor

Hexane Sol. (l)

H_2O Sol. (l)

If we wait long enough this system will come to equilibrium.

We find that there is
   some hexane in the water.
   some water in the hexane
   a mixture of water and hexane gas.

We know that the chemical potentials for each species in all phases of this system are equal.

The structure of the lipid system depends on the composition, the molecular details, the temperature and the pressure. In fact, the geometry of the lipid surface can be a very important energetic contributor to cellular chemistry.

It takes energy to form a surface into a shape.

For example, sheets of lipids take force both to compress and expand.

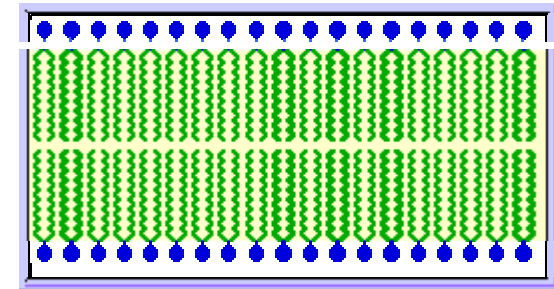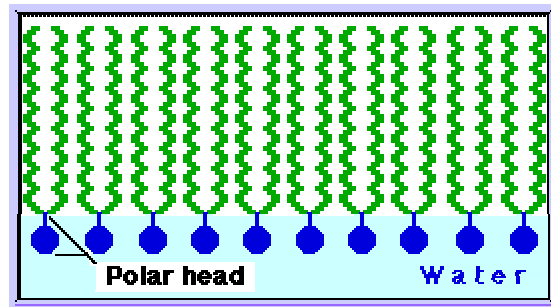This force and the related energy are measured using a Lanmuir film balance:

Polar head   Water

A   Pressure
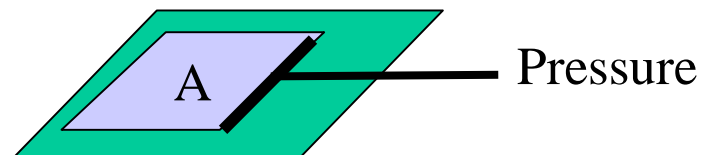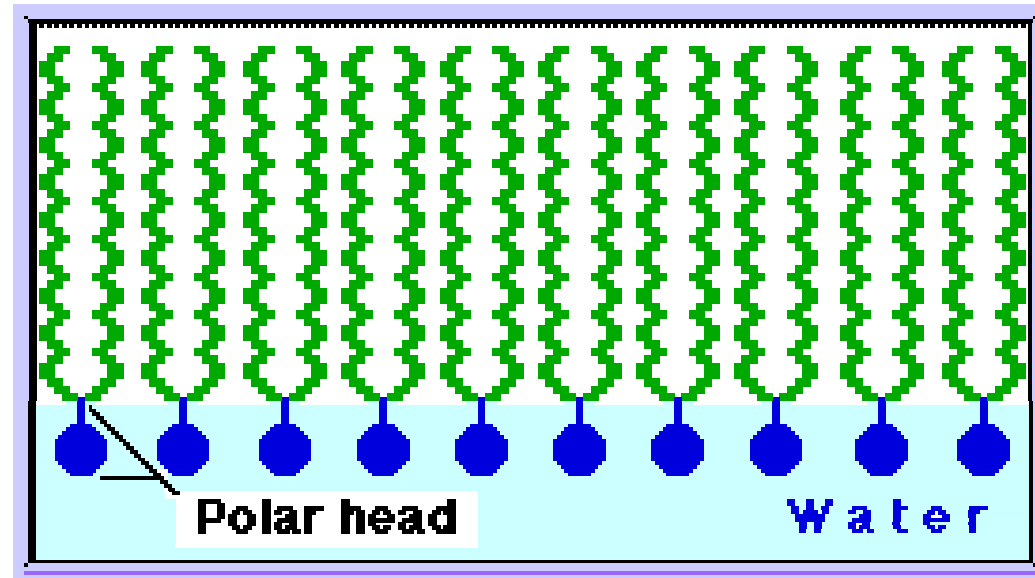
In cells and solutions lipids can be in many forms:

Monolayers

Bilayers

Micelles

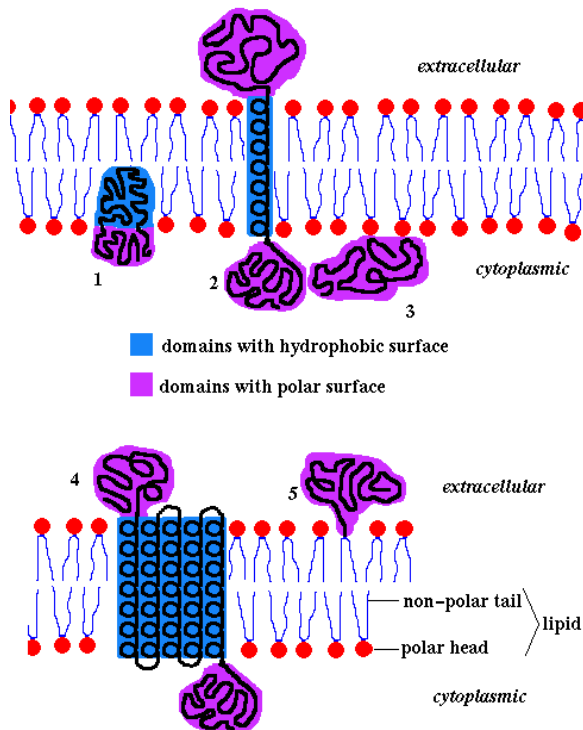Vessicles

Polar head   Water
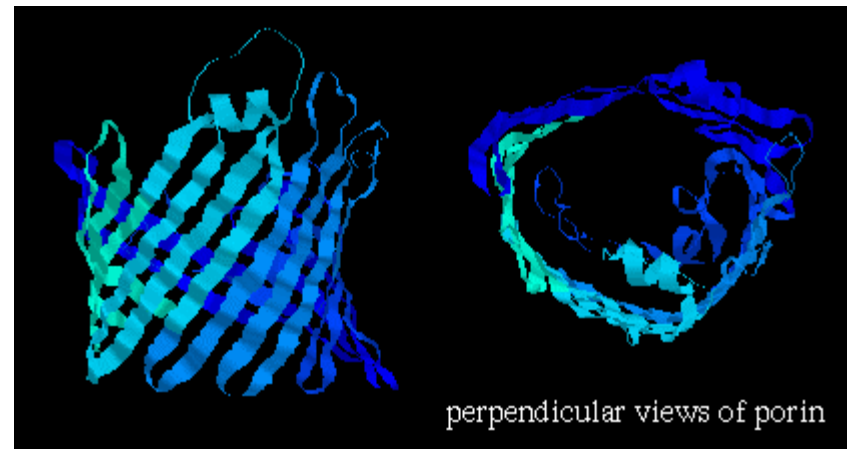
REVERSE MICELLE SOLUTION

Decane

AOT

Water

AOT:
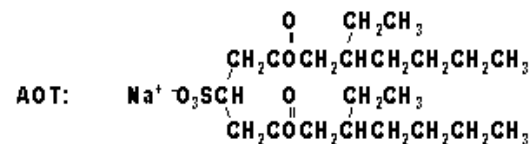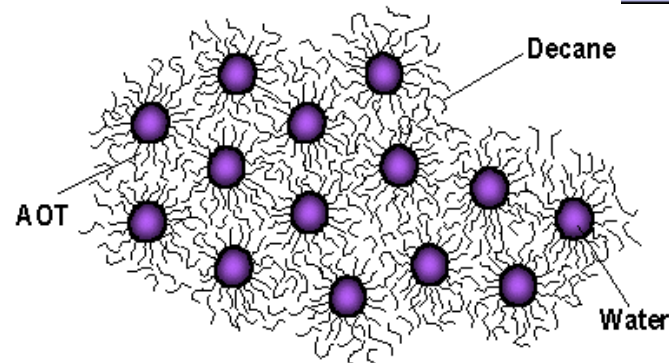
Macromolecular Interactions with Membranes

Proteins in the plasma membrane

domains with hydrophobic surface

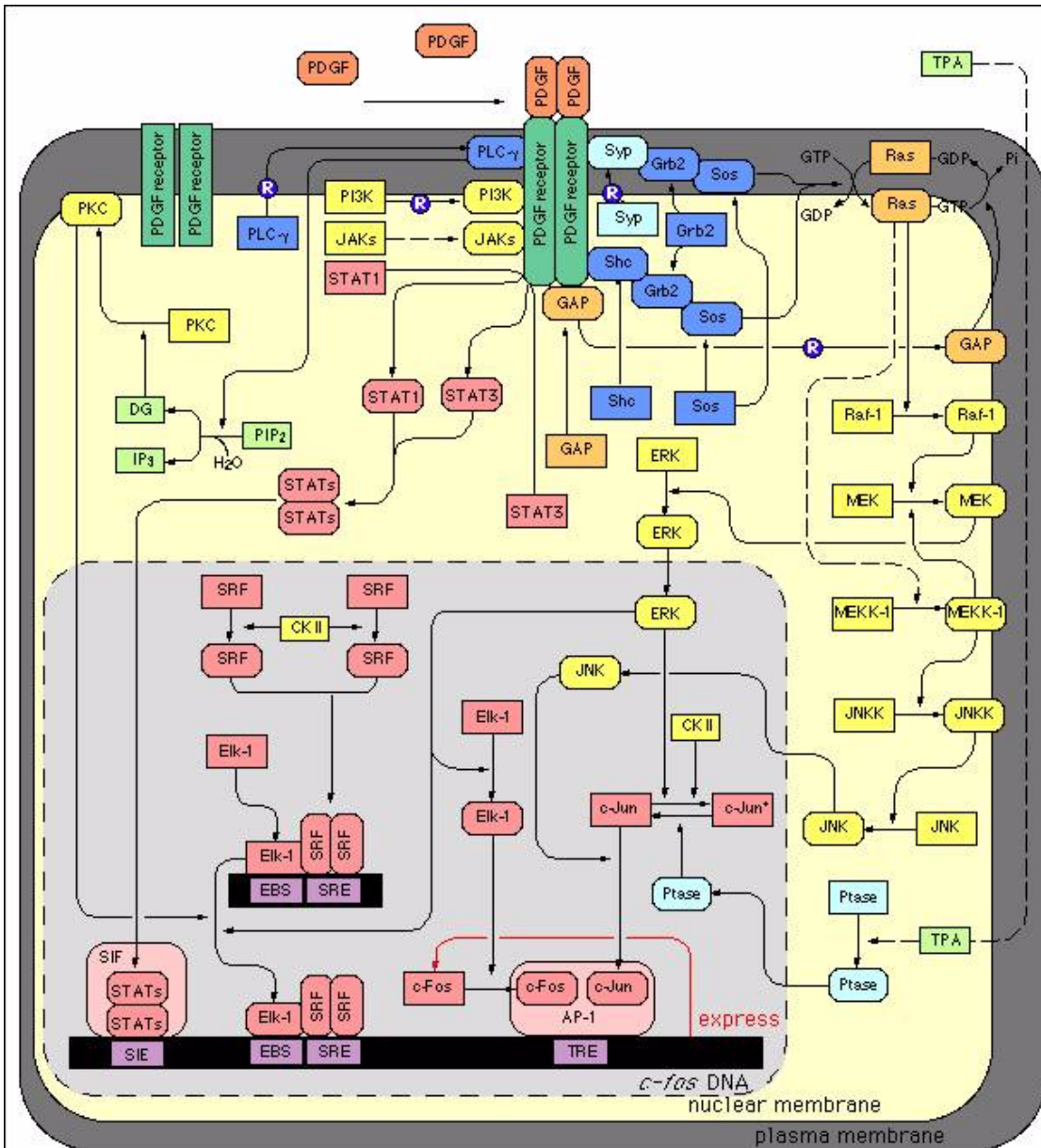domains with polar surface

File: Untitled1[Frame 1]   1 - 761
Table: Kyte & Doolittle
Window: 6   Average: -0.12   Threshold Line: 0.0

perpendicular views of porin
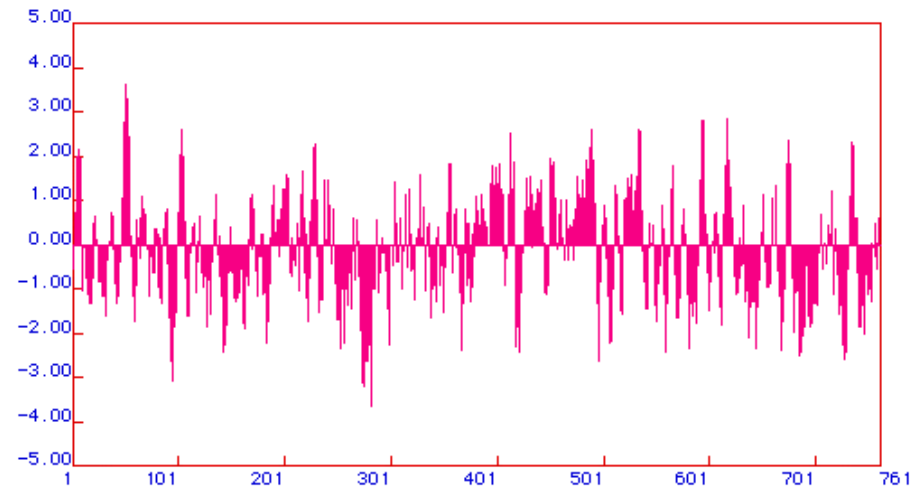
Platelet-Derived Growth Factor Pathway.

We were talking about

Physical Equilibria

Colligative Properties

The effect of binding on equilibria

Homework:

TSW 5.3(b,c),5.4,5.10,5.12,5.19,5.30